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ARTICLE

Protective effect of L-glutamine on lysosomal integrity in isoproterenol-induced myocardial infarction in rats

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ABSTRACT Lysosomal acid hydrolases play an imperative part in the initiation of inflammation processes associated with myocardial infarction. In the present investigation, we have studied the protective effect of L-glutamine on isoproterenol-induced myocardial damage in male albino rats with respect to alterations in lysosomal function. The myocardial infarction in experimental animals was induced by intraperitoneal (i.p.) injection of isoproterenol (11 mg/100 g body weight/day) for 2 days. The total and free activities of lysosomal acid hydrolases (β-glucuronidase, β-galactosidase, β-glucosidase and acid phosphatase) were determined in plasma and heart tissue of control and treated rats. Significant elevation in the total activities of lysosomal hydrolases was observed in plasma and heart tissue of isoproterenol administered rats. A parallel ($p<0.05$) rise in the free activities of these acid hydrolases in the cardiac tissue was also noticed. Isoproterenol-mediated lysosomal membrane fragility was evident from the altered subcellular distribution of heart β-glucuronidase activity. Prior oral administration of L-glutamine (100 mg/kg body weight/day for a period of 20 days) significantly attenuated the isoproterenol-induced release of these lysosomal hydrolases into the systemic circulation from the cardiac lysosomes and maintained the lysosomal stability at level comparable to that of control rats. The results of the present study suggested that the cardioprotective activity of glutamine might be related to its membrane-stabilizing property.
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KEY WORDS

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Introduction

Despite extensive advances in diagnosis and management over the last three decades, acute myocardial infarction continues to be a major public health problem (Boden 2003). A considerable body of experimental proof is now evolving which proposes that atypical discharge of lysosomal hydrolases plays a major role in the pathogenesis of myocardial infarction (Punithavathi and Prince 2010). Scientific knowledge on the events occurring in myocardial infarction condition has moved the cardiovascular research towards biomolecules, which can attenuate the incidence of myocardial ischemia. The efficacy of several cytoprotective agents on the prevention of necrotic injury connected with tissue damage has been accredited to their ability to preserve lysosomal integrity and to block the discharge of the lysosomal hydrolases (Jayachandran et al. 2009).

L-Glutamine is the most prevalent free proteinogenic aminoacid in many organs, including heart. Glutamine, traditionally considered a nonessential amino acid, now is considered "conditionally essential" after critical illness, stress, and injury. It plays an important role in the inter-organ nitrogen exchange and the maintenance of pH homeostasis (van de Poll et al. 2004). It is one of the major constituents of proteins and it functions as a chief metabolite in amino acid transamination reactions via α-ketoglutarate and glutamate. It delivers nitrogen for numeral biosynthetic pathways by serving as a major precursor of the purine and pyrimidine rings of nucleic acids and nucleotides including adenosine triphosphate (ATP) (Mora et al. 2002). It is used as an energy substrate in most cells, including heart myocytes (Suleiman et al. 1997). It is also involved in the regulation of endothelial nitric oxide metabolism in the cardiac tissue (Murphy and Newsholme 1998). Glutamine plays a vital part in reduced glutathione biosynthesis by providing glutamate to the glu-

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tathione antioxidant defense system (Roth 2008). Pisarenko and co-workers (1983) reported decline in the intracellular concentration of glutamine in the cardiac tissue during myocardial dysfunction. Even though glutamine is participating in numerous key biological and physiological functions in the myocardium; the cardioprotective activity of glutamine on lysosomal function in isoproterenol-induced myocardial infarction has not been studied in detail.

Isoproterenol [L-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], a β-adrenergic agonist, induces many morphologic and metabolic alterations in the heart tissue of experimental rats similar to those seen in myocardial infarction in man (Sathish et al. 2003; Anandan et al. 2013). It induces myocardial necrosis by a multiple step mechanism. It acts through β-adrenergic receptors, stimulating calcium influx, and augmenting the consumption of oxygen and depletion of ATP (Wallis et al. 2001). Lysosomal hydrolases play a major role in the pathogenesis of isoproterenol-induced myocardial infarction (Nirmala and Puvanakrishnan 1996). Alterations in lysosomal integrity and the activities of cardiac lysosomal hydrolases (β-glucuronidase, β-glucosidase, β-galactosidase and acid phosphatase) have been reported in isoproterenol-induced myocardial infarction (Prince and Priya 2010). One of the characteristic features of isoproterenol-induced myocardial infarction in experimental animals is the link between the progression of inflammatory responses and the extent of liberation of lysosomal hydrolases from the necrotic tissue into the systemic circulation (Ebenezer et al. 2003).

In this study, we have investigated the protective effect of L-glutamine on lysosomal function in isoproterenol-induced myocardial infarction in rats by virtue of its antioxidant (Marques et al. 2011) and membrane stabilizing properties (Anandan et al. 2013).

Materials and Methods

Chemicals

L-Glutamine, isoproterenol, p-nitrophenol, p-nitrophenyl β-D-galactopyranoside and p-nitrophenol-β-glucuronide were procured from Sigma (St. Louis. MO, USA). All the other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, weighing 120-150 g were used for the study. Animals were kept individually in polyurethane cages under hygienic and maintained standard environmental conditions (28±2 °C; humidity 60-70%; 12 h light/dark cycle). The control and treated rats were allowed free access to food (Sai Feeds, Bangalore, India) and water. The guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA; New Delhi, India) with the approval of Institutional Animal Ethics Committee (IAEC) were strictly followed during animal experiments.

Experimental protocol

The animals were divided into four groups, comprising six rats each.

Group I: Normal control rats received standard pelleted diet and intragastrically administered with distilled water for a period of 20 days and then injected with physiological saline alone for 2 days.

Group II: Rats intragastrically administered with L-glutamine (100 mg/kg body weight/day; dissolved in distilled water) for a period of 20 days and then injected with physiological saline alone for 2 days.

Group III: Rats administered with distilled water for a period of 20 days and then injected with isoproterenol (11 mg/100 g body weight/day dissolved in physiological saline, i.p. for 2 days for the induction of myocardial infarction.

Group IV: Rats pretreated with glutamine (100 mg/kg body weight/day, p.o for 20 days before the induction of myocardial infarction as described for Group III.

At the end of the experimental period (*i.e.* 24 h after last injection of isoproterenol), the control and treated rats were killed and blood was collected using EDTA as anticoagulant for the separation of plasma.

Separation of subcellular fractions

The hearts excised were immediately cut open and kept in isotonic saline to remove the blood. Then cardiac tissue samples were homogenized in 0.25 M ice-cold sucrose solution (0.5 g tissue/5 ml) at 4 °C. The plasma and a portion of myocardial homogenate were used for the determination of the total activity of lysosomal enzymes. The total activity in heart tissue referred to that detected in the 800 g supernatant fraction in the presence of 0.2% (v/v) Triton X-100, while the free activity referred to that detected in the 15 000 g supernatant without Triton X-100.

The remaining tissue homogenate portions were subjected to differential centrifugation and the different fractions were obtained as follows:

a) structural proteins, nucleus, and cell debris at 600 g for 10 min;

b) mitochondria at 5 000 g for 10 min;

c) lysosomes at 15 000 g for 10 min;

The nuclear and lysosomal fractions were then treated with Triton X-100 (final concentration 0.2% v/v) at 4 °C for 15 min just prior to the determination of enzyme activities.

Table 1. Total activity of lysosomal hydrolases (β-glucuronidase, β-galactosidase, β-N-acetyl glucosaminidase, acid phosphatase and cathepsin D) in plasma of control and treated groups of rats.

Parameters	Group I	Group II	Group III	Group IV
β-glucuronidase	2.50±0.15[a]	2.43±0.17 [a]	9.34±0.85 [b]	3.76±0.22[c]
β-galactosidase	1.42±0.09[a,c]	1.35±0.11 [a]	3.56±0.25 [b]	1.69±0.14 [c]
β-N-acetyl glucosaminidase	2.29±0.17[a]	2.34±0.15 [a]	3.89±0.21[b]	2.72±0.16[c]
acid phosphatase	1.56±0.12[a,c]	1.43±0.14 [a]	3.93±0.31 [b]	1.85±0.18[c]
cathepsin D	7.31±0.56[a]	7.18±0.61 [a]	21.4±0.19 [b]	10.25±0.92 [c]

Values that have a different superscript letter (a,b,c) differ significantly (p<0.05) with each other.

Table 2. Total activity of lysosomal hydrolases (β-glucuronidase, β-galactosidase, β-N-acetyl glucosaminidase, acid phosphatase and cathepsin D) in heart tissue of control and treated groups of rats.

Parameters	Group I	Group II	Group III	Group IV
β-glucuronidase	29.42±1.72[a]	27.36±1.61 [a]	39.25±2.38 [b]	31.01±1.99 [a]
β-galactosidase	9.36±0.69[a]	9.10±0.62 [a]	14.78±1.01 [b]	9.93±0.76 [a]
β-N-acetyl glucosaminidase	12.79±0.85 [a]	10.92±0.78 [b]	18.0±1.02 [c]	14.3±0.81 [a]
acid phosphatase	4.15±0.32 [a]	4.23±0.26 [a]	5.85±0.37 [b]	4.27±0.21 [a]
cathepsin D	19.36±1.57[a]	19.45±1.49 [a]	34.82±2.18 [b]	22.38±1.74 [a]

Values that have a different superscript letter (a,b,c) differ significantly (p<0.05) with each other.

Assay of lysosomal enzymes

The activities of β-glucuronidase and cathepsin D were determined according to the experimental procedures described by Devlin and Gianetto (1970) and Sapolsky et al. (1973), respectively. β-galactosidase and β-N-acetyl glucosaminidase were estimated by the method of Rosenblit et al. (1974) and acid phosphatase according to the procedure of King (1965). The subcellular localization (nuclear and lysosomal fraction) of β-glucuronidase was determined as a measure of lysosomal stability. The activities of β-glucuronidase, β-galactosidase and β-*N*-acetylglucosaminidase were measured as x10^{-2} μmoles of *p*-nitrophenol formed/h/mg protein. The cathepsin D activity was expressed as x10^{-3} μmoles of tyrosine liberated/h/mg protein. The acid phosphatase activity was quantified as x10^{-2} μmoles of phenol formed/min/mg protein.

Statistical analysis

Results are expressed as mean ± SD (standard deviation) for 6 animals. Multiple comparisons of the significant analysis of variance were performed by Duncan's multiple range comparison test. A P-value <0.05 was considered as statistically significant. All data were analyzed with the help of statistical package program, SPSS 10.0 for Windows.

Results and Discussion

Activities of total lysosomal hydrolases in plasma and heart tissue of control and treated rats are depicted in Table 1 and Table 2. In the present study, injection of isoproterenol resulted in a significant (p<0.05) elevation in the total activities of lysosomal enzymes (acid phosphatase, β-D glucosidase and β-D-galactosidase) in plasma and heart tissue of Group III rats compared to Group I control animals. A concomitant (p<0.05) rise in the free activities of these enzymes in the heart tissue was also noticed (Table 3). This is in agreement with an earlier report (Sathish et al. 2003). The observed cardiac lysosome fragility and increase in systemic acid hydrolases implies that outflow or extrusion of lysosomal hydrolases might be associated with stimulated endocytic processes harmful to cellular and subcellular membranes (Punithavathi and Prince 2010; Sathish et al. 2003). The rate of release of lysosomal specific β-glucuronidase is an index of lysosomal integrity. The altered subcellular distribution of β-glucuronidase in the heart tissue of isoproterenol-administered rats is an indication of instability of cardiac lysosomes (Table 4). The increased total and free activities of lysosomal acid hydrolases in the heart tissue of Group III rats confirms the increased expression of lysosomal hydrolases in isoproterenol-induced myocardial infarction.

Table 3. Free activity of lysosomal hydrolases (β-glucuronidase, β-galactosidase, β-N-acetyl glucosaminidase, acid phosphatase and cathepsin D) in heart tissue of control and treated groups of rats.

Parameters	Group I	Group II	Group III	Group IV
β-glucuronidase	15.27±1.12[a,c]	14.59±1.24[a]	27.34±2.46[b]	18.01±1.59[c]
β-galactosidase	6.51±0.47[a]	6.48±0.51[a]	10.28±0.89[b]	7.29±0.64[a]
β-N-acetyl glucosaminidase	8.75±0.63[a]	8.57±0.58[a]	14.82±1.14[b]	9.15±0.72[a]
Acid phosphatase	2.44±0.15[a,c]	2.23±0.19[a]	4.67±0.28[b]	2.74±0.23[c]
Cathepsin D	16.49±1.18[a]	15.91±1.24[a]	26.15±1.97[b]	18.18±1.51[a]

Values that have a different superscript letter (a,b,c) differ significantly ($p<0.05$) with each other.

Table 4. Subcellular localization of β-glucuronidase activity in heart tissue of control and treated groups of rats.

Parameters	Group I	Group II	Group III	Group IV
Nuclear fraction	4.98±0.31[a]	4.32±0.27[a]	6.93±0.51[b]	4.85±0.29[a]
Lysosomal fraction	24.51±1.14[a,c]	23.25±1.08[a]	32.17±2.15[b]	26.12±1.46[c]

Values that have a different superscript letter (a,b,c) differ significantly ($p<0.05$) with each other.

In Group IV animals, the administration of L-glutamine significantly inhibited the liberation of lysosomal hydrolases into the systemic circulation from the heart tissue. Glutamine might have exerted its protective actions either by modulating the lysosomal stability or by blocking the discharge of lysosomal hydrolases. Antioxidant defense of glutamine may protect the lysosomal membrane from isoproterenol-mediated reactive free radical species through the formation of reduced glutathione, which plays a major role in preserving the structural and functional integrity of the cellular and subcellular membranes (Roth 2008). Studies by Kadowaki and Kanazawa (2003) have shown that excess glutamine at tenfold the plasma level has an abnormal inhibitory effect on hepatic proteolysis, due to a lysosomotropic toxicity of ammonia derived from glutamine degradation. This amino acid is recognized at the plasma membrane, indicating the possible existence of glutamine receptor/sensor for its recognition and subsequent intracellular signaling. Investigations by Pourahmad and co-workers (2005) have indicated that glutamine administration attenuates the lysosomal hydrolases-mediated chromate cytotoxicity by acting as protease inhibitor and/or reactive oxygen species (ROS) scavenger.

Reports by Li and co-workers (2003) has indicated that adding a limited amount of glutamine to the freezing media is beneficial in maintaining the membrane and acrosome integrity in cryopreservation of cynomolgus monkey (*Macaca fascicularis*) sperm. Studies by Bergamini et al. (1993) have demonstrated the inhibitory activity of glutamine on macroautophagy and proteolytic function in liver of Sprague-Dawley albino rats with the use of the antilipolytic agent 3,5'-dimethylpyrazole as a stimulatory agent. Investigations by Pösö and co-workers (1987) have shown that glutamine and ethanol synergistically attenuate protein degradation in the liver by two discrete mechanisms: one decreasing the formation of autophagic vacuoles and the other involving lysosomotropic inhibition, possibly via ammonia. Studies by Mortimore and Pösö (1984) have indicated that of all the amino acids only glutamine directly exerts inhibitory activity on lysosomal pathways in hepatic protein degradation.

Conclusion

In conclusion, the results of the present investigation have demonstrated that glutamine possibly exerted its cardioprotective action through modulating structural and functional integrity of myocardial lysosomes. However, further investigations are essential to confirm the exact molecular mechanism of glutamine involved in the lysosomal membrane-stabilizing activity reported in this study.

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